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Artificial fertilization, embryonic and larval development of Hilsa, *Tenualosa ilisha* (Hamilton)

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ABSTRACT

Hilsa, *Tenualosa ilisha* is an important commercial fish of the Indo-Pacific region, especially in Bangladesh, India and Myanmar. Recently, catch of this species has declined due to overfishing and various ecological changes. In the present study, at first the embryonic and larval development of *T. ilisha* was studied in detail over 15 days. This was followed by examining the effects of different water sources on breeding performance. To obtain the gametes, migratory oozing male and female collected, bred through dry stripping ($n = 3$) and fertilized eggs were oxygen packed and transported to laboratory. The photomicrographs of all developmental stages of live embryo and larvae during incubation and after hatching were documented with the aid of a light microscope. Results demonstrated that morula, blastula, gastrula, neurula, somatogenesis and hatching began at 1:00, 3:30, 7:30, 11:30, 12:30 and 21:00 h post stripping (hps), respectively at 26 ± 0.91 °C water temperature. Heartbeat and muscular contraction of embryo commenced at 11:30 and 18:00 hps, respectively. The newly hatched larvae measured 2.50 ± 0.16 mm in total length with a yolk volume of 0.416 ± 0.157 mm^3. Key characters such as appearance of pectoral fin, opening of mouth, development of digestive system and anal pore of larvae were noticed on 2, 3, 5 and 8 dph, respectively. Yolk sac and oil globule were completely exhausted on 4th and 15th days post hatching (dph), respectively. Ten days old larvae commenced exogenous feeding. Breeding performance in three different sources of fresh-water, such as river water (RW), potable drinking water (PW) and freshwater from deep bore well (BW) was evaluated. Result of this study showed that the fertilization (34 ± 5.5%) and hatching rate (5 ± 1.5%) were significantly ($P < .05$) low in PW compared to RW and BW, due to low hardness and poor Ca^{+2} and Mg^{+2} ion concentration. Results of second experiment indicate that either filtered RW or BW is ideal for hilsa breeding. The present study will have substantial impact on development of aquaculture and conservation of hilsa.

1. Introduction

The Indian shad (*Tenualosa ilisha*), popularly known as hilsa, belonging to the family clupeidae, is widely distributed in Indo-Pacific region from the Arabian Gulf, along the coast of Pakistan, India, Bangladesh and Burma to South Vietnam (Bhaumik, 2015). It is a euryhaline, anadromous fish that inhabits freshwater rivers, estuaries and marine environments. It feeds and grows mainly in sea, and migrates to rivers for spawning; thereafter, juveniles develop and grow in fresh water, then migrate to ocean, where they spend most of their lives (Bhaumik, 2015; Sahoo et al., 2016; Hossain et al., 2019). It is an important commercial fish of the Indo-Pacific region, especially Bangladesh, India and Myanmar (Bhaumik, 2015; Rahman and Wahab, 2018; Hossain et al., 2019), where its fishery generates employment and income for millions of people, worth over US$ 2 billion (Sahoo et al., 2016). At present the total catch has declined due to obstruction of natural migration for breeding, over fishing, water pollution and sedimentation in rivers (BOBLME, 2014; Sahoo et al., 2016; Hossain et al., 2019). However, the annual global average catch shares of the *T. ilisha* of Bangladesh increased from 74.5% all through 1984–2013 to 86.7% during the 2010–2015 periods (Rahman and Wahab, 2018). For conservation and development of aquaculture of this species, numerous attempts on breeding through artificial fecundation and stripping from wild matured broodstock have been made since 1908 to till 1962 (Sahoo et al., 2016), however they fail to rear the larvae. Later on Malhotra et al. (1969), Malhotra et al. (1970), Mathur et al. (1974) and Bhanot and De (1984), could rear the larvae in pond system. Recently, larval rearing of hilsa in tank system is carried out by Chattopadhyay

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et al. (2019), they found that the shape of tank, stocking density and water depth have a significant effect on survival of hilsa larvae. Their study demonstrated that the stocking density of 300 nos m^{-3}, water depth of 0.962 m and circular shape tank are ideal for hilsa nursery. Only few published literatures on embryonic and larval development of hilsa through camera lucida drawing were reported (Kulkarni, 1950; Jones and Menon, 1951; De and Sen, 1988. So far, there are no published reports on detailed ontogeny of embryonic and larval development of hilsa till oil globule utilization through digital photography.

Ideal physico-chemical parameters and ionic composition of water are essential for success of embryonic development of teleost eggs (Van der Velden et al., 1991). Many studies have shown that total hardness has significant influences on fish eggs fertilization (Ofor and Udeh, 2012), hatching and larval survival (Molokwu and Okpokwasili, 2002; Townsend et al., 2003). With this background, embryonic and larval developments of hilsa were studied in detail over 15 days with the aid of light microscopy. This was followed by an experiment to assess the effect of different sources of fresh water such as river water (RW), bore well water (BW) and potable drinking water (PW) on breeding performance of hilsa.

This study will aid in understanding the chronological developmental stages of embryo and larvae of hilsa. Further, suitable ionic composition of water for breeding will be understood. Overall, study will help in conservation and development of captive breeding programme of hilsa.

2. Materials and methods

2.1. Embryonic and larval development

2.1.1. Collection of mature broodstock

Mature male and female fish during its spawning migration were captured using gill net from the Hooghly River at Godakhali, West Bengal, India (Lat. 23° 44′ 17″ N and Longitude 86° 20′ 55″ E) for artificial breeding.

2.1.2. Selection of oozing male and female

Male (Mean total length: 248 ± 18 mm; mean total weight: 246 ± 39 g) with white colour oozing milt, and female (mean total length: 430 ± 75 mm; mean total weight: 782.5 ± 93.94 g) with light yellow oozing eggs, were selected for artificial breeding. A total of four breeding attempts were made between February to March 2018, viz. 16th February, 27th February, 3rd March and 6th March 2018. In each breeding attempts, three breeding trials were made ($n = 3$) to estimate the mean value of relative fecundity, diameter of eggs, fertilization rate, hatching rate, incubation period and to observe the chronological development of embryo and larvae.

2.1.3. Fecundation and fertilization through dry stripping

Fertilization of eggs was carried out through dry stripping method (Malhotra et al., 1969, 1970; Mathur et al., 1974; Sen et al., 1990). In brief, oozing female and male in sex ration of 1:3, were collected live, wiped out with tissue paper to remove water from body surface. Thereafter, female was stripped to collect eggs in a dry steel bowl and milt from three males was striped directly on the eggs (Fig. 1a). Both eggs and milt were mixed gently with a sterile bird feather. Clean filtered and aerated bore well water (BW) was sprinkle over the egg-milt mixture and mixed gently. Five to six times washing at an interval of five minutes were carried out to remove extra milt and ovarian fluid. After one h of post stripping (hps), approximately 50 thousands eggs were oxygen packed in polythene bags. Each bag having capacity of 25 L was filled with five litre of BW and medical grade oxygen, and transported to Kakdwip Research Centre (KRC) of ICAR-Central Institute of Brackishwater Aquaculture (CIBA) in air conditioned vehicle in 3.30 to 4.00 h.

2.1.4. Breeding performance

Breeding performance was assessed by calculating relative fecundity, fertilization rate, incubation period and hatching rate as followed:

Weight of stripped eggs (g) = Weight of female before stripping (g) – Weight of female after stripping (g)

Number of eggs stripped (g) = Weight of stripped eggs (g) × number of eggs g^{-1} of egg sample

Relative fecundity = Number of eggs stripped / weight of the female (g)

Fertilization and hatching percentages were estimated for each female (100 eggs from each female) using the following formulae:

Fertilization rate (%) = 100 × (number of fertilized eggs / total number of eggs counted). This was determined at 30 min post stripping.

Hatching rate (%) = 100 × (number of eggs hatched / total number of fertilized eggs). This was determined 45 min after inception of hatching.

2.1.5. Incubation and embryonic development

After three to four hour of road transportation, eggs were carefully shifted from oxygen packed polythene to the glass jar (20*L*) filled with BW. Stocking density of eggs in each glass jar was nearly 25,000 eggs L^{-1}. To avoid water temperature fluctuation, incubation was carried out in air conditioned room (Temperature at 26 °C) at normal photoperiod. In order to examine the embryonic development, 30 ± 10 eggs were collected from the incubation jar at an interval of 30 min until hatching. Embryonic development photographs were captured under a trinocular microscope (Radical RXLr-5, India) at 4× and 10 X magnifications. All the measurements of embryo were carried out through image-analysing software (ProgRes CapturePro 2.7). Further, heart beats, somatic movement and twitching movement were recorded at every minute.

2.1.6. Larval development

Immediately after hatching, 30 ± 5 larvae were randomly collected, measured and photographed under the trinocular microscope. Larvae were carefully transferred to larval rearing tank (5000 L) filled with filtered aerated fresh-water. Larval development was monitored at 12 h intervals to till yolk sac and oil globule utilization. Measurement of total length (TL), mouth gap, yolk sac, oil globules were carried out with the image-analysing software. The yolk sac volume (V) was calculated as V = p/6 *Lh2 where, 'h' is yolk sac height and 'L' is yolk sac length (Korzelecka-Orkisz et al., 2010). After yolk sac utilization, larval rearing tanks were inoculated with algae (*Chlorella spp*) at a density of 1–5 × 10^3 cells mL^{-1} to maintain good environmental condition.

2.2. Effect of different water sources on breeding performance

2.2.1. Breeding of Hilsa in three different freshwater sources

This experiment was conducted to evaluate the ideal fresh water for breeding of hilsa. In this experiment, three separate breeding trials at an interval of one day were conducted ($n = 3$) with three different sources of freshwater such as river water (RW), potable drinking water (PW) and freshwater from deep bore well (BW) to evaluate breeding performance of hilsa. Breeding was carried out following the earlier protocol of experiment one.

2.2.2. Trace metal analysis

Trace metals, such as Na, Mg, K, Ca, Mn and Fe concentrations in all the three different sources of freshwater such as RW, PW and BW were analysed following the methodology described by Neeraj et al. (2017). In brief, water samples were filtered with 0.45 mm size filter papers,

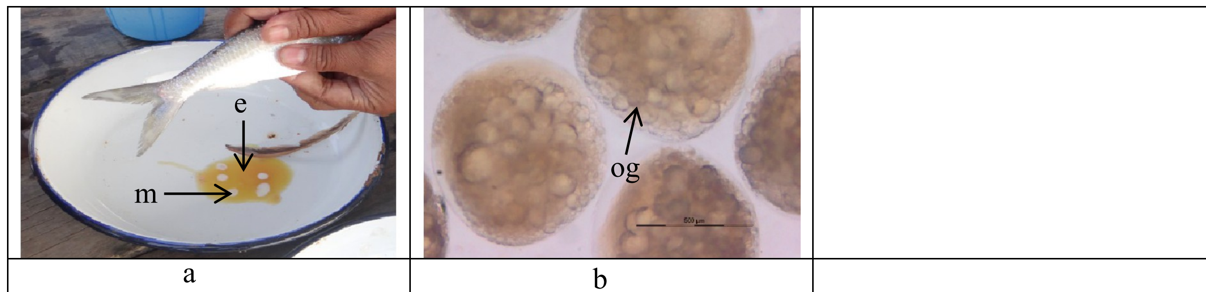

Fig. 1. Stripping of ripe male and female *T. ilisha*. (a) stripped eggs with numerous oil globules; (b) fertilized eggs. (e-eggs; m- milt; og- oil globule).

filtrates were made acidic with 100 mL of pure HNO_3 (69%, Himedia Laboratory Pvt. Ltd., Mumbai, India) and proceeded for trace elements analysis through Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) (Agilent 7700 series, Agilent Technologies, USA).

2.2.3. Physico-chemical parameters

Three different sources of freshwater such as RW, PW and BW used for breeding trail were collected to measure different physico-chemical parameters, namely temperature, pH, dissolved oxygen (DO), conductivity, salinity, RH, total alkalinity and total hardness. Temperature, pH DO, salinity, conductivity and RH value were measured with probe (HACH-HQ40d). Total hardness and alkalinity were measured following standard methodology of APHA, 1995.

2.3. Data analysis

Mean and standard deviation for all the data were calculated using MS-Excel. Comparison of all the variables between treatments in the experiment was executed through one-way analysis of variance (ANOVA). The analysis was carried out using SPSS-20 for windows.

3. Results

3.1. Fecundation and breeding

Data on female body weight, relative fecundity, oocyte diameter, fertilized egg diameter, perivitelline space, fertilization rate, hatching rate of all the breeding trials are summarized in Table 1. Oozing male body weight ranged from 245 to 385 g with the mean of 295 ± 53.37 g. The mature stripped eggs were brown, demersal, spherical (mean diameter: 769.04 ± 8.36 μm) and filled with numerous oil globules (Fig. 1b).

3.2. Embryonic development and organogenesis

Embryonic stage started immediately after fertilization and lasted till hatching out from the chorion. Summarized embryonic and larval developmental stages of *T. ilisha* with an average time period and key characters are mentioned in Table 2.

3.2.1. Fertilized eggs

Fifteen minutes after stripping fertilized and unfertilized eggs were distinguishable. Fertilized eggs were transparent, buoyant and non-adhesive in nature (Fig. 2a). Mean diameter, perivitelline space and yolk sac diameter of fertilized eggs were 2.03 ± 0.10 mm, 0.634 ± 0.083 and 0.738 ± 0.02 mm, respectively (Table 1 and Fig. 2c). The fertilized egg blastodisc was filled with yolk and five to eight small oil globules, and one large oil globule (Fig. 2c). Fertilized eggs were, easily buoyed with slight agitation of water, whereas unfertilized eggs were opaque, white in colour and floating on water surface (Fig. 2b).

3.2.2. Morula

One hps, fertilized eggs attained morula stage (Fig. 2d).

3.2.3. Blastula

Three distinct sub-stages of blastula were noticed such as early, mid and late blastula. Early blastula was characterized by non-synchronous cell division and uncountable number of blastomere cells (Fig. 2e). Mid blastula was characterized by flattening of blastodermal cells, formation of epithelial layer, which is termed as enveloping layer (EL) and this covered the underlying blastomeres (Fig. 2f). In late blastula, yolk syncytial layer (YSL), a cellular structure at the junction of blastomeres marginal layer and yolk sac was present (Fig. 2g). During this stage, number of oil globules reduced and a blastocoel formed inside the blastodisc. At the end, EL started spreading towards vegetal parts, marking the onset of epiboly; which indicates the transitional stage from late blastula to the start of gastrula.

3.2.4. Gastrula and neurula

Onset of epiboly started the gastrulation. Epiboly is the morphogenetic peristaltic cellular movement occurred on the yolk surfaces, which allows the embryo to move within the perivitelline spaces. Epiboly leads to formation of germ ring with outer epiblast and lower hypoblast. The epiboly of 30%, 50%, 70%, and 90% is shown in Fig. 2h, i, j and m, respectively. After 70% of epiboly, formation of germ ring was noticed (Fig. 2k). At this stage, local accumulation of cells at one place along germ ring was visible, which is known as embryonic shield (Fig. 2l). Prior to the completion of epiboly, lump of yolk cell beyond the blastoderm margin was noticed as yolk plug (Fig. 2m). Closer of blastopore marked the end of peristaltic cellular movement, epiboly and/or gastrulation and establishment of embryonic axis. During this stage, tail bud and polster (cephalic bud) was discernible, which is known as neurula stage of the development (Fig. 2n).

3.2.5. Somatogenesis and organogenesis

Somatogenesis is the formation and development of somite blocks or miotomes at the cephalic parts of the embryo and their progress towards caudal region. Embryo with 4–5, 12–13, 15–20 and 23–25 somites are shown in Fig. 2o, p, q and r, respectively. At 38–40 miotomes stage, Kupffer cells, eye bud, otic capsules or auditory vesicle with otolith, distinguished forebrain, midbrain and hindbrain were observed (Fig. 2s, t and u). After 18 hps, detachment of caudal from yolk sac begun and first muscular contraction in embryo was noticed. Muscular twitching was at the rate of 6–8 min^{-1}. During 19 hps, two chambered heart, complete detachment of tail from yolk, nutrient flow from yolk sac to hepatocytes were noticed (Fig. 2v). During 21 hps, vigorous muscular twitching and lashings of the detached free-end of caudal part against the chorion were observed (Fig. 2w). At the time of hatching, in embryo having 40–45 miotomes, heart rate and caudal movement were 80 ± 8.16 and 22 ± 3.65 min^{-1}, respectively. Embryo started hatching out of chorion after an incubation period of 21 ± 1.63 hps at water temperature of 26 ± 0.91 °C. During hatching, tail came out first (Fig. 2x) and hatching completed in 30 min.

Table 1
Details of breeding trials (n = 3) that include female body weight (g), relative fecundity (numbers of eggs/ g weight of female), mature oocyte diameter (μm), fertilized egg diameter (mm), perivitelline space (mm), fertilization rate (%), hatching rate (%), incubation period (h), size of hatching (mm) and water temperature (°C).

Breeding trial	Female body weight	Weight of stripped eggs	Number of eggs stripped	Relative fecundity	Mature oocytes diameter	Fertilized egg diameter	Fertilization rate	Perivitelline space	Hatching rate	Incubation period	Size of hatching	Water temperature
I	670 ± 12	70.50 ± 3.4	315,225 ± 120	470.48 ± 2.3	767.5 ± 9	1973 ± 13	87 ± 1.3	0.745 ± 0.01	60 ± 2.7	21:00 ± 0.15	2.50 ± 0.05	25.5 ± 2.3
II	780 ± 10	70.00 ± 2.6	245,000 ± 80	314.10 ± 1.5	759.9 ± 5.3	2200 ± 65	76 ± 2.3	0.653 ± 0.02	72 ± 9.2	23:00 ± 0.20	2.34 ± 0.04	25.00 ± 2.0
III	780 ± 7.5	80.00 ± 2.8	288,000 ± 45	369.23 ± 2.7	780.00 ± 3.5	2000 ± 70	73 ± 2.5	0.576 ± 0.01	68 ± 2.4	21:00 ± 0.10	2.46 ± 0.05	26.5 ± 1.5
IV	900 ± 2.5	120.00 ± 3	504,000 ± 78	560.00 ± 2.5	769.00 ± 2.4	1978 ± 41	84 ± 1.3	0.563 ± 0.02	81 ± 2.6	19:00 ± 0.10	2.73 ± 0.04	27.00 ± 1.0
Mean ± SD	782.5 ± 93.94	80.01 ± 23.79	338,056 ± 313-2.44	428.45 ± 109.-01	769.04 ± 8.36	2.03 ± 0.10	80 ± 6.58	0.634 ± 0.083	70.25 ± 8.73	21.00 ± 1.63	2.50 ± 0.16	26 ± 0.91

Values are expressed as mean ± standard error (SE). Values in a column does not vary significantly (p > .05).

3.3. Larval development

The newly hatched larvae were comma shaped, and transparent, with a large oval shaped yolk sac and oil globules. One larger oil globule was present at centre of yolk sac and 4 to 6 small oil globules were at posterior or anterior end of yolk sac (Fig. 3a). Yolk sac was segmented in nature. Details of larval development from 0 days post hatching (dph) to 15 dph are narrated in Table 3. Larvae were photophilic in nature.

3.4. Breeding performance

Breeding performance in terms of fertilization rate ranged from 73 to 87% with a mean value of 80 ± 6.58%. The incubation period varied from 19 to 23 h at respective water temperature of 27 to 25.5 °C. Mean incubation period was 21 ± 1.63 h at water temperature of 26 ± 0.91 °C. Average hatching percentage was 70.25 ± 8.73.

3.5. Second experiment

3.5.1. Physico-chemical parameters and ionic composition of water

Physico-chemical parameters, such as pH, temperature, salinity, conductivity, DO, total hardness, calcium hardness, magnesium hardness and ionic composition of different source of freshwater are summarized in Table 4. PW had significantly lower concentration of Na^{+}, Ca^{+2} and Mg^{+2} ions, and calcium hardness than that of BW and RW.

3.5.2. Breeding performance

Fertilized egg diameter was significantly higher (P < .05) in PW (2300 ± 0.03 mm) than BW (2100 ± 0.02 mm) and RW (2050 ± 0.01 mm) groups. Similarly, perivitelline space was larger in PW group (0.09 ± 0.01) than other two groups. Fertilization and hatching rate were significantly (P < .05) low in PW compared to RW and BW. Average fertilization rates (%) of 34 ± 5.5, 75 ± 11.5 and 82 ± 7.2 were noticed in PW, BW and RW, respectively. Similarly, hatching rates (%) were 5 ± 1.5%, 72 ± 9.5 and 78 ± 11.5 in PW, BW and RW, respectively. Shorter incubation period of 18 ± 0.30 hps was observed in PW than BW and RW (21:00 ± 0.10 hps) at an average water temperature of 26 °C. Total length of newly hatched larvae was significantly smaller in PW groups (1.6 ± 0.04 mm) than other two groups (2.4 ± 0.05 and 2.3 ± 0.03 mm).

4. Discussion

The peak breeding season of hilsa in Hooghly river system is during February to March and July to October (Bhaumik and Sharma, 2012). In both trials, breeding was carried out on full moon and new moon days of February to March in the afternoon or evening when water temperature was low (25.5 to 27 °C). Similarly, De (1980) reported that the most favourable time for artificial breeding during afternoon or evening when the water temperature was 24 to 29 °C. Knowledge on embryonic and larval development helps in the successful large-scale rearing of larvae. Embryonic development is a complex process in which cellular differentiation and proliferation occur concurrently though their rate is different (Hall, 1922). Diameter of mature stripped eggs/oocytes was 769.04 ± 8.36 μm, fifteen minutes after fertilization fertilized egg swelled (diameter of 2.03 ± 0.10 mm), with perivitelline space of 0.634 ± 0.083 mm. De, 1986 reported that average diameter of mature eggs at the time of stripping ranged from 760 to 870 μm, whereas fertilized eggs after swelling attained average diameter of 2.02 mm (De, 2014) and 2.1 to 2.3 mm De and Sen (1988). The difference in stripped mature egg diameter might be due to the quality of broodstock. A healthy mature broodstock accumulates more yolk in the oocytes and thereby having larger egg size (Bromage and Roberts, 1995).

In present study, we observed that the fertilized eggs were greenish-

Table 2
Key morphological characteristics and average timing at embryonic developmental stage of hilsa, *Tenulalosa ilisha* (water temperature: 26 ± 0.91 °C).

Developmental stages	Average time period in hour post stripping, hps (h: min.)	Specific characters
Fertilized eggs	00:00	Spherical in shape
Morula	01:00	Blastodisc with many blastomere cells on animal pole
Blastula		
Early blastula	03.30	Uncountable number of blastomere cells
Mid blastula	04.00	Enveloping layer covering the blastomeres
Late blastula	06.30	Yolk syncytial layer, germ ring and blastocoel formed
Gastrula		
30% epiboly	07:30	Germ ring epiboled 30% of yolk sac
50% epiboly	08:30	Germ ring epiboled 50% of yolk sac
70% epiboly	09:30	Germ ring epiboled 70% of yolk sac, embryo formation
90% epiboly	10:15	Germ ring epiboled 90% of yolk sac, yolk plug formation
Neurula	11:30	Tail bud and polster (cephalic bud) discernible
Somatogenesis		
4–5	12:30	Neurocoele cell or brain vesicle
12–13	14:00	Optic vesicle, notochord and first hear beat
15–20	15:00	Brain formed
23–25	17:00	Brain differentiation evident
38–40	18:00	Eye bud, auditory vesicle with two otoliths, distinguished forebrain, midbrain and hindbrain. Embryo first muscular contraction noticed. Detachment of caudal part from yolk sac began.
Hatching	21:00	Hatching completed in 30–40 min

yellow, demersal and buoyed with slight agitation of water which swelled and turned transparent after 15 min of fertilization, whereas unfertilized eggs turned white and swelled larger. Similar observation was made by De and Sen (1988). Perivitelline space filled with fluid cushions that protect embryo from mechanical injury and help in osmoregulation (Buzollo et al., 2011). In hilsa, we have measured the perivitelline space of 0.063 ± 0.008 mm, which is very close to the earlier report (0.07 to 0.08 mm) of De (2014). Cleavage in fertilized eggs of hilsa is meroblastic type, where 4-cell, 8- cell, and 16 cell stage reached by 55 min, 1: 15 h, 1:30 h after fertilization (De, 1914). In present study, cleavage of embryo could not be observed due to transportation of fertilized eggs to laboratory. In morula stage, numerous blastomeres are produced, which is referred as mulberry, half-berry or ball-like shape (Honji et al., 2012; Olaniyi and Omitogun, 2013). In this study, one hps fertilized eggs attained morula stage, which was characterized by the presence of numerous uniform sized blastomere cells on blastodisc. Contrary to this, De (1986) reported that hilsa embryo attained morula stage 3:30 h after fertilization. Shape and size of blastomeres of hilsa were uniform, which is similar to other teleosts (Hall, 2008). In teleost, blastula occurred at eight zygotic cycles or 128-cell stage before the onset of gastrulation (Kimmel et al., 1995). In the current study, blastula was characterized by the formation of YSL at 4.00 to 6.30 hps through covering of blastomere cells by EL. We also noticed spreading of EL towards vegetal pole at 6: 30 hps, which marked the onset of epiboly and transition from blastula to gastrula stage. In the same species, during blastula stage, flattening of blastodem cells on yolk mass after 5:30 h after fertilization is reported by Kulkarni (1950) and De (1986). In teleosts, transition from blastula to the beginning of gastrula is marked by emergence of epiboly and the completion of gastrulation is marked by the closure of blastopore (Buzollo et al., 2011; Kimmel et al., 1995). In hilsa, morphogenetic peristaltic cellular movement occurs on the yolk surface during 7:30 hps, which is known as epiboly, and marked the inception of gastrulation and closer of blastopore by 11:00 hps indicating the end of gastrulation. There is no detailed literature available in this species to support this finding. However, De (2014) has reported the gastrula and yolk plug stage at 5:30 and 8.00 h after fertilization, respectively. Olaniyi and Omitogun (2013) reported polster and tail bud as markers for the identification of cranial and caudal parts, respectively, during neurulation. In our study, neuralation was characterized by the formation of tail bud and polster (cephalic bud) during 11:30 hps. Early segmentation was characterized by the formation of brain vesicle, optic vesicle, notochord and first heart beat during 12:30 to 17: 00 hps, and later segmentation was distinguished by discernibility of Kupffer cells, eye bud, otic capsules or auditory vesicle with otolith, distinguished forebrain, midbrain and hindbrain, which is similar to the observation made by Kimmel et al., 1995; Buzollo et al., 2011; Honji et al., 2012 and Olaniyi and Omitogun, 2013 in other teleost. Hilsa embryo at 18:00 hps was characterized by the formation of somite block and two chambered heart. At this stage, inception of muscular contraction and detachment of tail bud from yolk were also seen. Earlier reports of De and Sen (1988) and De (1986) in hilsa demonstrated the formation of optic vesicles (18 myotomes) and auditory capsule during 10:15 and 13:00 h after fertilization, respectively. The Kupffer vesicle is an important character of teleost embryo (Cardoso et al., 1995) and it indicates the allantoic rudiment (Kimmel et al., 1995). Kupffer vesicle has a role in the development of brain, heart and gut in zebrafish embryo (Essner et al., 2005). In this study, mean incubation period of the fertilized eggs was 21.00 ± 1.63 h at an ambient water temperature of 26 ± 0.91 °C, which is different from the reports of Kulkarni (1950), and De and Sen (1988). They found an incubation period of 18–26 h at 27.5–29.5 °C (Kulkarni, 1950) and 16–20 h at 24–29 °C (De and Sen, 1988) in the same species. The hatching in fish is facilitated by muscular contraction or twitching at caudal part (Honji et al., 2012; Olaniyi and Omitogun, 2013). In this study, vigorous movement of tail broke down perivitelline membrane and hatchling emerged out as tail first in most of the cases, which is similar to the observation made in other fishes, such as common carp *Cyprinus carpio*, barb *Barbus barbus* and rainbow trout *Oncorhynchus mykiss* (Ługowska and Sarnowski, 2011), *Heteropneustes fossilis* (Puvaneswari et al., 2009) and *Mystus gulio* (Kumar et al., 2018). However, De (2014) reported that in hilsa, during hatching the head comes out first from the egg. Similarly, Langeland and Kimmel (1997) opined that during hatching, head comes out first and tail comes out last from the embryo in most of the teleost. We observed the number of myotomes in embryo during hatching ranged from 40 to 45, which is almost similar to the earlier report of De and Sen (1988) who noticed 40 myotomes. Hatching period depends on fertilization period, faster the fertilization shorter the hatching period and survival of the embryos (Olaniyi and Omitogun, 2014). In this study, hatching period for hilsa was 30 min, whereas De (1986) and De and Sen (1988) reported hatching period of 3 h in hilsa (hatching starts 18 h after fertilization and completed by 21 h after fertilization). The newly hatched larvae of hilsa were transparent, devoid of any pigmentation and had large yolk sac with five to six oil globules as energy reserved, which is similar to

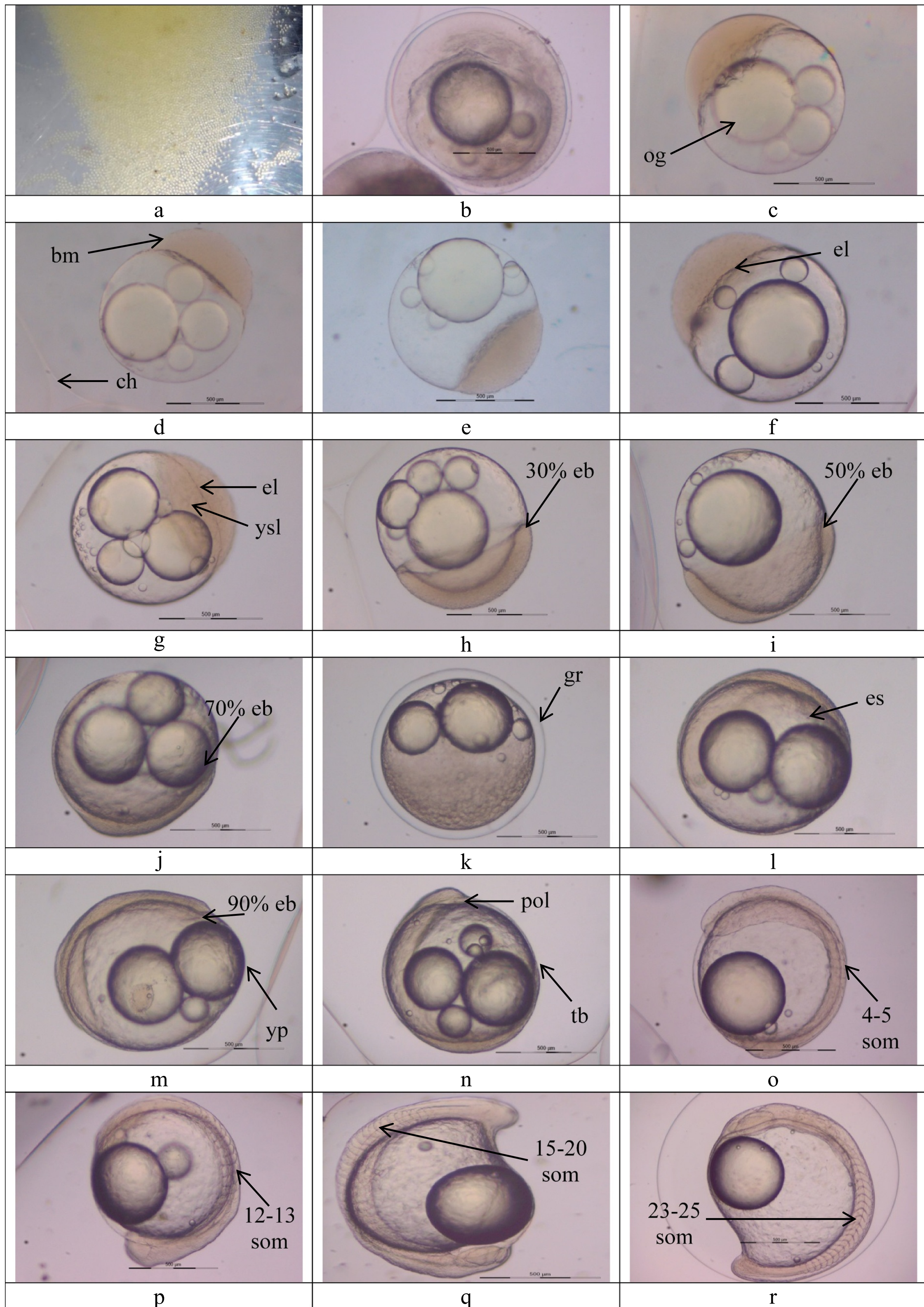

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Fig. 2. Embryonic development of *T. ilisha* at 26 ± 0.91 °C. (a) fertilized eggs; (b) unfertilized eggs; (c) fertilized eggs; (d) morula; (e) early blastula; (f) mid blastula; (g) late blastula; (h) 30% epiboly; (i) 50% epiboly; (j) 70% epiboly; (k) germ ring; (l) embryonic shield; (m) 90% epiboly (yolk plug stage); (n) neurula; (o) 4–5 somites; (p) 12–13 somites; (q) 15–20 somites; (r) 23–25 somites; (s) 38–40 miotomes; (t) Kupffer cells and eye lens; (u) auditory vesicle; (v) embryo, 19 hps (solid arrow showing heart and broken arrow indicate bold flow); (w) embryo, 21 hps; (x) hatching. (og-oil globule; bm- blastomeres; el- enveloping layer; ysl- yolk syncytial layer; eb- epiboly; gr- germ ring; es embryonic shield; yp- yolk plug; pol-polster; tb- tail bud; som- somites; fb- fore brain; mb- mid brain; hb- hind brain; kc- Kupffer cells; eyl- eye lens; oto- otolith; ch- chorion; t- tail).

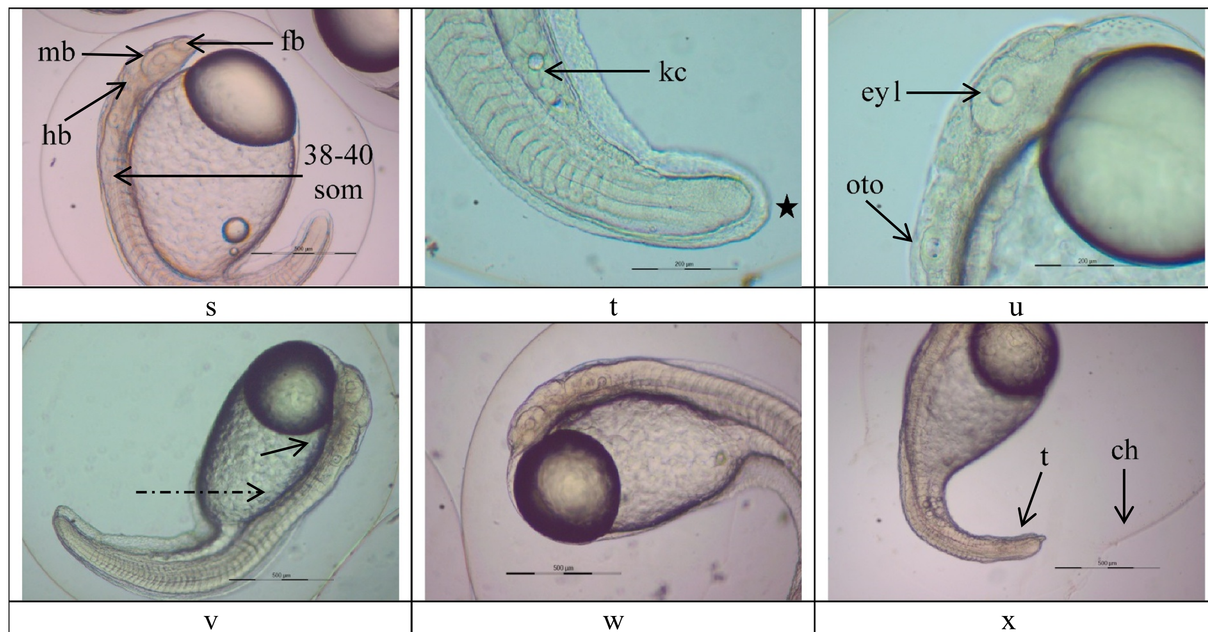

Fig. 2. (*continued*)

the other fish larvae (Bjelland and Skiftesvik, 2006) and hilsa (Kulkarni, 1950 and De and Sen, 1988). In present study, size of 0, 1, 2, 3, 4 and 5 dph larvae was 2.50 ± 0.16, 3.94 ± 0.109, 4.45 ± 0.22, 4.71 ± 0.67, 4.71 ± 0.67, 5.58 ± 0.84 mm, respectively which is nearly same as earlier observation of De and Sen, 1988 (2.41, 3.75, 3.97, 4.15, 4.42 and 5.12 mm).We could count the number of myotomes in 0, 1, 2 and 3 dph larvae as 40–45, 45–48, 80–100 and 100–110, respectively which differ from earlier observation of De and Sen (1988) who reported number of myotomes of one day to five day old larvae varied from 49 to 56 only. De and Sen (1988) observed development of pectoral bud on 2nd dph, active swimming and opening of mouth on 3rd dph, pigmentation of eye on 4th dph and caudal fin rays formation on 5th dph. In present study, pectoral fin bud was seen on 2nd dph, opening of mouth, development eye and active swimming started from 3rd dph; yolk sac utilized at 4th dph; developed digestive system with stomach, intestine and liver was seen at 5th dph and anal pore opened at 8th dph. After above preparedness, larvae started exogenous feeding from 10th or 11th dph and oil globule exhausted on 15th dph at water temperature of 26 ± 0.91 °C. Therefore, hilsa larvae started exogenous feeding after complete exhaustion of yolk sac and before oil globule utilization. Water quality variables, such as temperature (22–29 °C), dissolved oxygen (6.5–8.2 ppm), pH (7.6–7.8) for hilsa breeding and egg incubation were reported by De (2014). In present study, BW was used, which has an average temperature, pH, salinity, conductivity and dissolve oxygen of 28 ± 1.3 °C, 6.82 ± 0.04, 0.25 ± 0.01 gL^{-1}, 1134 ± 1.5 μs/ cm, 6.10 ± 0.82 ppm, respectively.

Ionic composition of river water, drinking water and bore well water vary because of difference in bedrock geology, land use, human activities and rainfall (Zhu et al., 2012). The American Society of Engineers classifies water of 0–60, 61–120, 121–180, and above 180 $mg\,L^{-1}$ CaCO3 total hardness as soft, moderately hard, hard, and very hard respectively. Moderately hard water had a positive influence, while hard water had a negative influence on hatching rate of eggs of *Rhamdia quelen* (Silva et al., 2003). In the present study, fertilization and hatching rates were significantly poor in PW compared to RW and BW due to low calcium (70 $mg\,L^{-1}$ $CaCO_3$) and total hardness (100 $mg\,L^{-1}$ $CaCO_3$), which would have reduced egg turgor and increased mechanical injury. Similar observation was made by Ketola et al. (1988) in Atlantic salmon, *Salmo salar*; rainbow trout, *Onchorhynchus mykiss;* brook trout, *Salvelinus fontinalis* where they recommended minimum calcium hardness of 64 $mg\,L^{-1}$. We also noticed that fertilized eggs swelled, perivitelline space increased and burst before hatching in PW due to low hardness. Similar observation was made in silver carp, *Hypophthalmichthys moltrix*; striped bass, *Morone saxatilis* and common carp, *Cyprinus carpio* eggs incubated in soft water, which absorbed excess water and burst prematurely (Gonzal et al., 1987; Van der Velden et al., 1991 and Silva et al., 2005). Absorption of excess water by fertilized eggs in PW also leads to shorter incubation period and hatching out of pre-mature smaller size larvae from embryo. There is no published literature to support the current finding and it is needed to conduct further detailed study in this area.

In addition, imbalance between Ca—Mg ratios adversely affects embryonic development, larval growth and survival (Brown and Lynam, 1981; Silva et al., 2003; Wang et al., 2002; Si et al., 2016). In this study Ca—Mg ratio of 0.91 significantly reduced fertilization and hatching rate. Sodium is known to regulate osmotic and ionic gradient of egg plasma membrane and absorption of some ions by egg from ambient water (Alderdice, 1988). Low fertilization and hatching in PW would have been caused by osmotic imbalance resulted from the significantly low concentration of Na^{+} ions.

5. Conclusion

For the first time, we have investigated the chronological developmental stages during ontogeny and organogenesis of hilsa, *T. ilihsa*

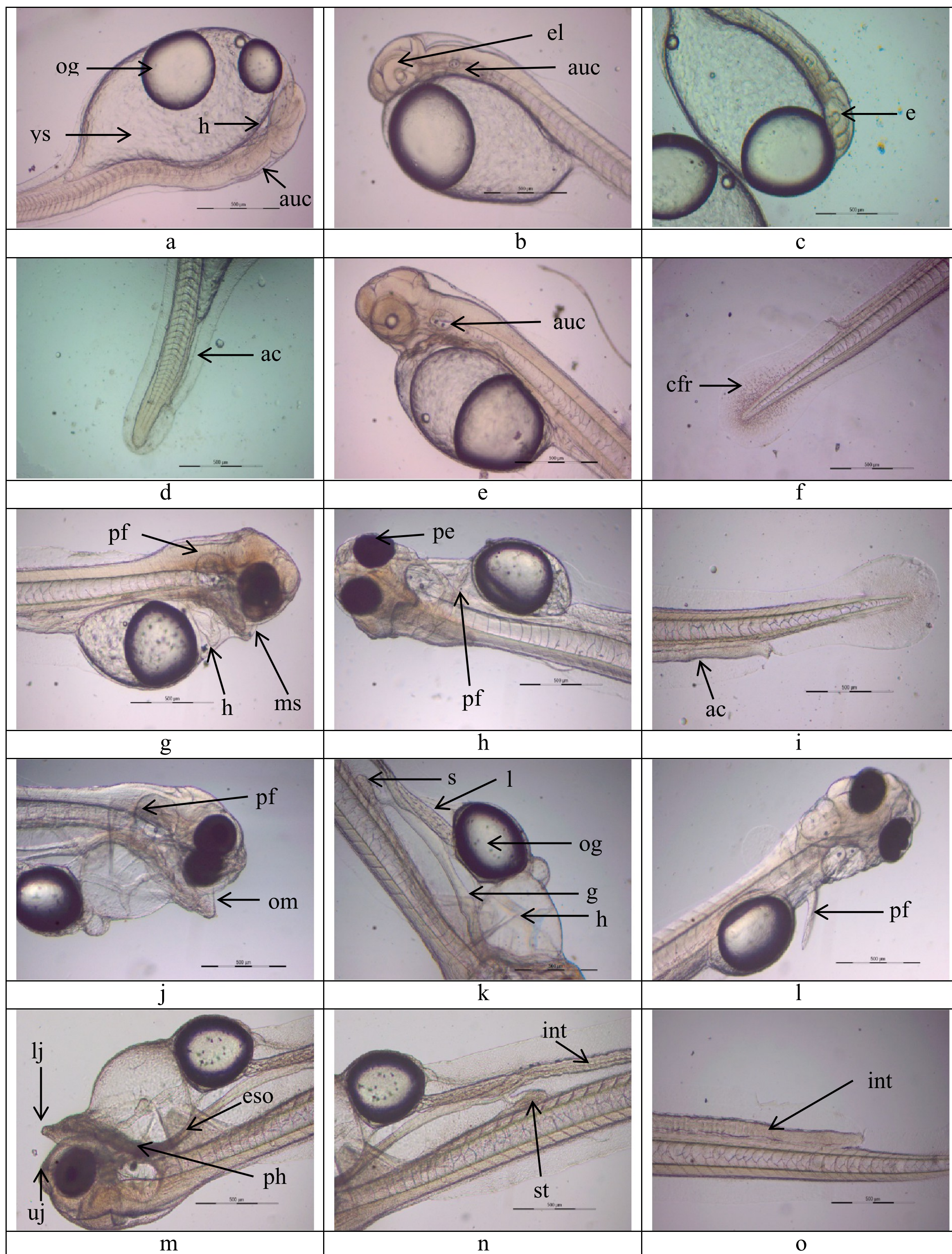

Fig. 3. Larval development of *T, ilisha.* (a) newly hatched larvae; (b) 12 h old larvae; (c) 1 dph larvae; (d) 1 dph larvae-rudimentary alimentary canal; (e) 30 h old larvae-eye lens (f); 30 h old larvae-caudal fin rays; (g) 2-dph larvae; (h) 3 dph larvae; (i) 3 dph larvae-gut; (j) 4 dph larvae; (k) 4 dph larvae-preliminary stomach and liver; (l) 4 dph larvae with developed pectoral fin.; (m) 5 dph larvae- jaws; (n) 5 dph- larvae-digestive system (o) 5 dph larvae- coiled intestine (p) 5 dph larvae-caudal fin rays; (q) 6 dph larvae- pigmentation in gut; (r) 6 dph larvae-rectum; (s) 7 dph larvae- pectoral fin rays; (t) 8 dph larvae- anal pore opened; (u) 9 dph larvae-upper and lower lip; (v) 10 dph larvae- few algal cells; (w) 11 dph larvae- many algal cells; (x) 12 dph larvae- gill arch; (y) 12 dph larvae- with many algal cells; (z) 13 dph larvae- little oil globule; (za) 14 dph larvae- scars of oil globule; (zb) 15 dph larvae- oil globule exhausted. (og- oil globule; ys- yolk sac; h- heart; el- eye lens; auc-auditory capsule; e- eye; ac- alimentary canal; cfl- caudal fin rays; pf- pectoral fin; h- heart; ms- mouth slit; pe- pigmented eye; om- open mouth; s- stomach; l- liver; g-gut; lj- lower jaw; uj- upper jaw; int- intestine; pig- pigmentation; rec- rectum; pfr- pectoral fin rays; ap- anal pore; ul- upper lip; ll- lower lip; gl- gill; oge- oil globule exhausted).

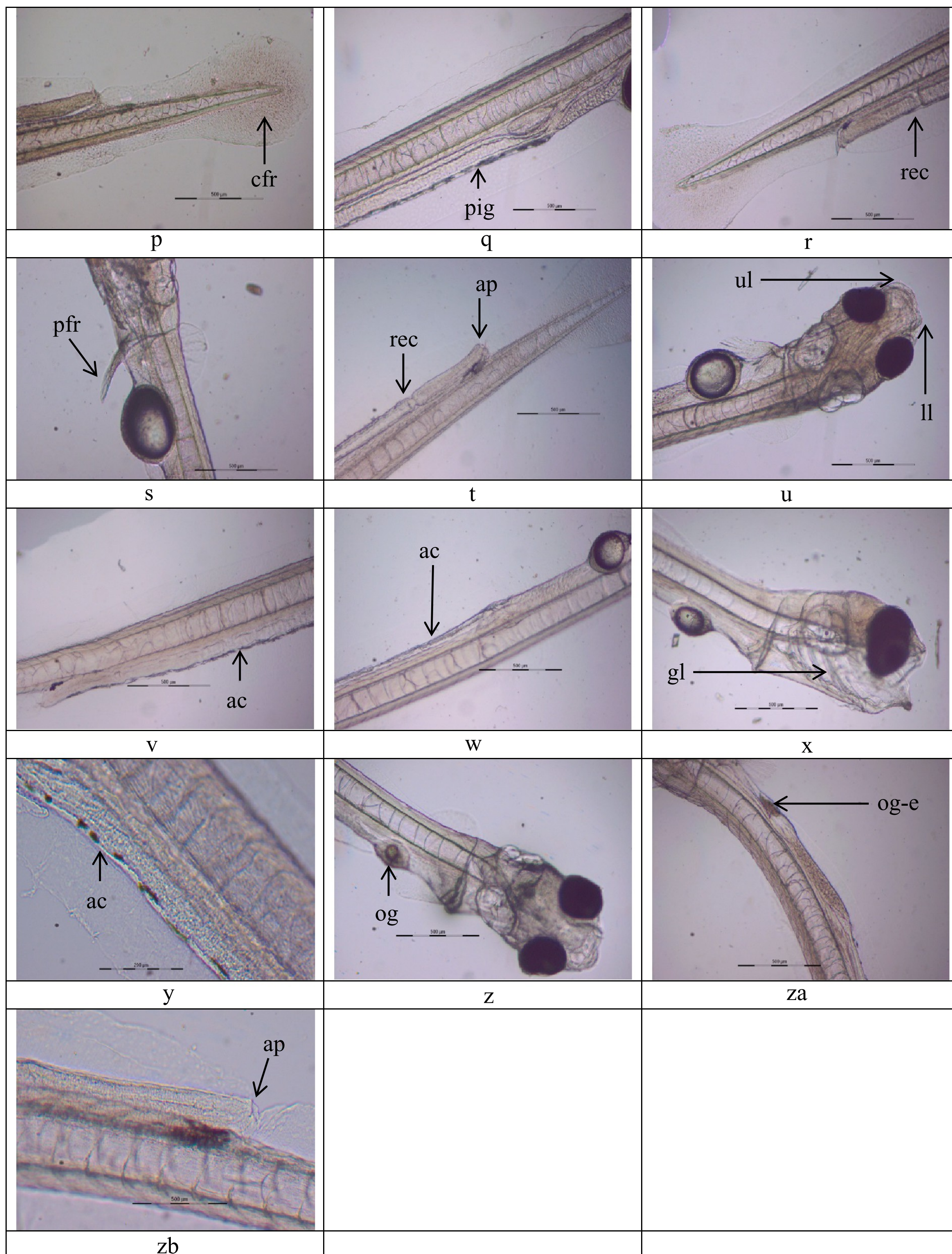

Fig. 3. (*continued*)

Table 3
Total length (mm), yolk sac volume (mm^3), oil globule diameter (mm), mouth gap (μm) and key morphological character of *Tenualosa ilisha* larval development from 0 to 15 days post hatching (dph).

Age of larvae	Total length	Yolk sac volume	Oil globule diameter	Mouth gap	Specific characters
0 dph	2.50 ± 0.16	0.416 ± 0.157	0.438 ± 0.008	Closed	Tubular heart, well differentiated brain (forebrain, midbrain and hindbrain), otic capsule with two otolith, un-pigmented eyes, 40 to 45 miotomes and round caudal fin margin (Fig. 3a). In 12 h old larvae, otic capsules moved towards hind brain, larger oil globule shifted from centre of yolk sac to its anterior end and eye lens appeared (Fig. 3b).
1 dph	3.94 ± 0.109	0.196 ± 0.036	0.420 ± 0.048	Closed	Eye without pigmentation (Fig. 3c), rudimentary alimentary canal tube seen (Fig. 3d) and a total of 45 to 48 miotomes were counted.
2 dph	4.45 ± 0.22	0.098 ± 0.041	0.413 ± 0.023	Mouth slit	In 30 h old larvae, caudal fin rays were visible and eyes with clear lens. (Fig. 3e and f). Two chamber heart, mouth slit, pectoral fin bud, partial pigmented eye, melanophores spot on yolk sac were seen. A total of 80 to 100 myotomes counted (Fig. 3g).
3 dph	4.71 ± 0.67	0.06 ± 0.01	0.411 ± 0.06	71 ± 0.13	Larvae swam actively from bottom to column. The otic capsule closer to the lens placode, both eyes were completely pigmented, both upper and lower jaws formed, mouth opened (Fig. 3h), and gut formed (Fig. 3i), miotomes counted from 100 to 110.
4 dph	4.71 ± 0.67	Nil	0.362 ± 0.04	164.8 ± 3.51	Yolk utilized, mouth widely opened, rudimentary alimentary canal with pharynx, esophagus and digestive tube (Fig. 3j), elementary stomach and liver noticed (Fig. 3k), well-developed pectoral fin (Fig. 3l).
5 dph	5.58 ± 0.84	Nil	0.344 ± 0.03	171.61 ± 1.96	Well-developed and elongated lower jaw and pharynx (Fig. 3m), digestive system has developed stomach, intestine and liver (Figs. 3n), coiled intestine (Fig. 3o), caudal fin with fin rays (Fig. 3p).
6 dph	5.87 ± 0.43	Nil	0.326 ± 0.002	173.02 ± 1.05	Pigmentation noticed on ventral side of intestine (Fig. 3q), formation of rectum with slight congestion near hind gut was noticed (Fig. 3r).
7 dph	5.88 ± 0.12	Nil	0.322 ± 0.03	174.71 ± 2.55	Pectoral fin rays developed (Fig. 3s)
8 dph	5.91 ± 0.05	Nil	0.312 ± 0.02	177.07 ± 1.09	Eight days old larvae showed opening of anal pore (Fig. 3t)
9 dph	5.94 ± 0.22	Nil	0.275 ± 0.04	178.99 ± 4.22	Lower and upper lip developed (Fig. 3u).
10 dph	5.98 ± 0.12	Nil	0.261 ± 0.03	182.15 ± 1.88	Prominent coiled intestine with few algal cells (Fig. 3v).
11 dph	5.98 ± 0.13	Nil	0.138 ± 0.01	185.06 ± 0.96	Algal cells predominant in gut (Fig. 3w).
12 dph	6.01 ± 0.39	Nil	0.118 ± 0.01	188.89 ± 1.20	Gill arch developed (Fig. 3x), algal cells were prominent in gut (Fig. 3y)
13 dph	6.01 ± 0.01	Nil	0.079 ± 0.02	195.74 ± 7.24	Very little oil globule and sensitive to handling (Figs. 3z).
14 dph	6.04 ± 0.27	Nil	Not measurable	207.9 ± 6.52	Scars or very little oil globule was visible on fourteen days, which was not measurable (Fig. 3za)
15 dph	6.06 ± 0.24	Nil	Nil	209.88 ± 13.16	Oil globule exhausted completely on 15th days and anal pore was completely open (Fig. 3zb).

Values are expressed as mean ± standard error (SE); dph, days post hatching.

Table 4
Physico-chemical parameters and ionic composition of three different sources of fresh water viz. potable drinking water (PW), bore well water (BW) and river water (RW).

Water sample	Potable drinking water (PW)	Bore well water (BW)	River water (RW)
Water quality			
Temperature (°C)	$28^a \pm 1.2$	$28^a \pm 1.3$	$26^b \pm 0.5$
pH	$7.62^a \pm 0.10$	$6.82^a \pm 0.04$	$7.82^a \pm 0.55$
Salinity (ppt)	$0.02^c \pm 0.01$	$0.25^b \pm 0.01$	$0.67^a \pm 0.01$
Conductivity (µs/cm)	$122.3^c \pm 5.0$	$1134^b \pm 1.5$	$1370^a \pm 3.6$
DO ($mg\,L^{-1}$)	$5.82^b \pm 0.3$	$6.10^b \pm 0.82$	$8.82^a \pm 1.0$
Total hardness ($mg\,L^{-1}$)	$100^c \pm 16.8$	$220^b \pm 5.60$	$260^a \pm 11.20$
Calcium hardness ($mg\,L^{-1}$)	$70^c \pm 8.50$	$126^a \pm 13.30$	$142^a \pm 10.20$
Magnesium hardness ($mg\,L^{-1}$)	$183^c \pm 2.40$	$210^a \pm 2.30$	$153^b \pm 1.80$
Ionic composition (µg/l)			
Na	$3139.41^c \pm 0.48$	$27,298.86^b \pm 1.40$	$31,616.99^a \pm 1.39$
K	$854.39^b \pm 0.81$	$999.79^b \pm 0.74$	$1253.98^a \pm 0.69$
Ca	$1235.05^c \pm 0.42$	$4455.67^b \pm 0.19$	$5109.64^a \pm 0.63$
Mg	$1345.53^c \pm 0.14$	$6608.04^a \pm 1.10$	$5893.85^b \pm 1.18$
Mn	$2.02^b \pm 1.23$	$9.12^a \pm 1.03$	$2.71^b \pm 1.24$
Fe	$49.58^b \pm 0.70$	$109.15^a \pm 0.35$	$59.28^b \pm 2.32$
Ca/Mg ratio	0.91	0.67	0.86

Values are expressed as mean ± standard error (SE). Values in a row with different superscript (a, b and c) differ significantly ($p < .05$).

through digital photomicrographs, which will be useful in artificial propagation of this species. Further, the detailed information on larval development will help in developing feed and feeding strategies of hilsa during its larval rearing. We also investigated that freshwater with total hardness and calcium hardness < 100 and $70\,mg\,L^{-1}$, respectively is not ideal for artificial propagation of hilsa. The data from the present study will contribute to the understanding of developmental research of hilsa for aquaculture and conservational researches.

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References

Alderdice, D.F., 1988. Osmotic and ionic regulation in teleost eggs and larvae. In: Hoar, W.S. (Ed.), An Arid Environment from Watersheds in Northern Xinjiang. 153. Geomorphology (Amst), China, pp. 102–114.
APHA, 1995. Standard Methods for the Examination of Water and Wastewater, 19th ed. American Public Health Association and Water Pollution Control Federation, Washington.
Bhanot, K.K., De, D.K., 1984. Observations on culture of *Hilsa ilisha* (Hamilton) in a freshwater pond. In: Proc. 71. Indian Sci, Congo. (Ranchi).
Bhaumik, U., 2015. Prospective of reproductive biology and spawning behaviour of Indian Shad (*Tenualosa ilisha*): a global review. Int. J. Curr. Res. Acad. Rev. 3, 229–241.
Bhaumik, U., Sharma, A.P., 2012. Present Status of hilsa in Hooghly-Bhagirathi river. CIFRI Bull. 179, 42.
Bjelland, R.M., Skiftesvik, A.B., 2006. Larval development in European hake (*Merluccius merluccius* L.) reared in a semi intensive culture system. Aquac. Res. 37, 1117–1129. https://doi.org/10.1111/j.1365-2109.2006.01536.
BOBLME, 2014. Report of the Hilsa Fisheries Assessment Working Group Meeting, 26–28 November 2014, Kolkata, India, BOBLME-2014-Ecology. 14. pp. 1–20.
Bromage, N., Roberts, R.J., 1995. Broodstock management and seed quality-general considerations. In: Bromage, Roberts (Eds.), Broodstock Management and Egg and Larval Quality. Blackwell Science, pp. 424.
Brown, D.J.A., Lynam, S., 1981. The effect of sodium and calcium concentrations on the hatching of eggs and the survival of the yolk sac fry of brown trout, *Salmo trutta L* at low pH. J. Fish Biol. 19, 205–211.
Buzollo, H., Veríssimo-Silveira, R., Oliveira-Almeida, I.R., Alexandre, J.S., Okuda, H.T., Ninhaus-Silveira, A., 2011. Structural analysis of the *Pimelodus maculatus* (Lac_ep_ele, 1803) embryogenesis (Siluriformes: Pimelodidae). Neotrop. Ichthyol. 9, 601–616. https://doi.org/10.1590/S1679-62252011000300013.
Cardoso, E.L., Alves, M.S.D., Ferreira, R.M.A., Godinho, H.P., 1995. Embryogenesis of the Neotropical freshwater siluriform *Pseudoplatystoma corruscans*. Aquat. Living Resour. 8, 343–346. https://doi.org/10.1051/alr:1995037.
Chattopadhyay, D., Chakraborty, A., Ray, P.K., Mandal, R., Banik, S.K., Suresh, V.R., Ghosh, K., 2019. Larval rearing of Hilsa shad, *Tenualosa ilisha* (Hamilton 1822). Aquac. Res. 50 (3), 778–785. https://doi.org/10.1111/are.13934.
De, K.K.Maturity, 1980. fecundity and spawning of post-monsoon run of Hilsa, Hilsa (Hamilton) in the upper stretches of the Hooghly estuarine system. J. Inland Fish Soc. India. 12 (1), 54–63.
De, D.K., 1986. Studies on the food and feeding habit of Hilsa. In: *Hilsa ilisha* (Ham.) of the Hooghly Estuarine System and Some Aspects of its Biology. University of Calcutta, pp. 285 PhD thesis.
De, D.K., 2014. The Shad Hilsa, *Tenualosa ilisha* (Hamilton) its Biology and Fisheries. Narendra Publishing House, Delhi, India, pp. 227.
De, D.K., Sen, P.R., 1988. Observations on the embryonic and early larval development of Indian shad, *Tenualosa ilisha* (Ham.). J. Inland Fish. Soc. India 18, 1–12.
Essner, J.J., Amack, J.D., Nyholm, M.K., Harris, E.B., Yost, H.J., 2005. Kupffers vesicle is a ciliated organ of asymmetry in the zebrafish embryo that initiates left-right development of the brain, heart and gut. Development 132, 1247–1260. https://doi.org/10.1242/dev.01663.
Gonzal, A.C., Aralar, E.V., Pavico, J.M.F., 1987. The effects of water hardness on the hatching and viability of silver carp (*Hypophthalmichthys molitrix*) eggs. Aquaculture 64, 111–118.
Hall, B.K., 1922. Evolutionary Development Biology. Chapman and Halll, London, UK.
Hall, T.E., 2008. Pattern formation. In: Finn, R.N., Kapoor, B.G. (Eds.), Fish Larval Physiology. Science Publishers, Enfield, New Hampshire, pp. 3–25.
Honji, R.M., Tolussi, C.E., Mello, P.H., Caneppele, D., Moreira, R.G., 2012. Embryonic development and larval stages of *Steindachneridion parahybae* (Siluriformes: Pimelodidae) - implications for the conservation and rearing of this endangered Neotropical species. NeotropicalIchthyology 10 (2), 313–327. https://doi.org/10.1590/S1679- 62252012005000009.
Hossain, M.S., Sharifuzzaman, S.M., Rouf, M.A., et al., 2019. Tropical Hilsa shad (*Tenualosa ilisha*): biology, fishery and management. Fish Fish. 9 (20), 44–65. https://doi.org/10.1111/faf.12323.
Jones, S., Menon, P.M.G., 1951. Observations on the life history of the Indian shad *Hilsa ilisha* (Ham.). Proc. Natl. Acad. Sci. 31 (3), 101–125.
Ketola, H.G., Longacre, D., Greulich, A., Phetterplace, L., Lashomb, R., 1988. High calcium concentration in water increases mortality of salmon and trout eggs. Progr. Fish Culturist. 50, 129–135.
Kimmel, C.B., Ballard, W.W., Kimmel, S.R., Ullmann, B., Schilling, T.F., 1995. Stages of embryonic development of the Zebrafish. Dev. Dyn. 203, 253–310. https://doi.org/10.1002/aja.1002 030302.
Neeraj, Kumar, Krishnani, K.K., Meena, K.K., Gupta, S.K., Singh, N.P., 2017. Oxidative and cellular metabolic stress of *Oreochromis mossambicus* as biomarkers indicators of trace element contaminants. Chemosphere 17, 265–274. https://doi.org/10.1016/j.chemosphere.2016.12.066.
Kulkarni, C.V., 1950. Breeding habits, eggs and early life history of the Indian shad, *Hilsa ilisha* (Ham.) in the Narmada River. Proc. Natl. Acad. Sci. 15, 169–176.
Korzelecka-Orkisz, A., Smaruj, I., Pawlos, D., Robakowski, P., Tanski, A., Szulc, J., Formicki, K., 2010. 2010. Embryogenesis of the stinging catfish, Heteropneustes follilis (Actinopterygii. Siluriformes: Heteropneustidae). Acta Ichthyologica et piscatorial 40 (2), 187–197.
Kumar, P., Biswas, G., Ghoshal, T.K., Kailasam, M., Vijayan, K.K., 2018. Embryonic and larval developments of brackish water catfish, *Mystus gulio* (Hamilton and Buchanan, 1822) induced with human chorionic gonadotropin and consequent larval rearing. Aquac. Res. 49 (7), 2466–2476. https://doi.org/10.1111/are.13706.
Langeland, J., Kimmel, C.B., 1997. The embryology of fish. In: Gilbert, S.F., Raunio, A.M. (Eds.), Embryology, Constructing the Organism, pp. 383–407.
Ługowska, K., Sarnowski, P., 2011. Heads or tails - fish hatching. Acta Ichthyol. Piscat. 41, 13–17. https://doi.org/10.3750/AIP2011.41.1.03.
Malhotra, J.C., Mathur, P.K., Kamal, M.Y., Chandra, R., Desai, V.R., 1969. Successful

artificial propagation of *Hilsa ilisha* (Ham.) near Allahabad. Curr. Sci. 38, 429–430.
Malhotra, J.C., Mathur, P.K, Kamal, M.Y., Mehrotra, S.N., 1970. Observations on the hatching of fertilized eggs of Hilsa ilisha (Ham.) in confined freshwater. Current Science 39, 538–539.
Mathur, P.K., Malhotra, J.C., Mehrotra, S.N., Shah, K.L., 1974. Experiments on the nursery rearing of spawn of *Hilsa ilisha* (Hamilton) in freshwater ponds. J. Inland Fish. Soc. Ind. 6, 205–210.
Molokwu, C.N., Okpokwasili, G.C., 2002. Effect of water hardness on egg hatchability and larval viability of *Clarias gariepinus*. Aquacult. Int. 10, 57–64.
Ofor, C.O., Udeh, H., 2012. Effect of water hardness on fertilisation and hatching success of *Clarias, gariepinus.,* (Burchell, 1822). *Heterobranchu,s longifilis* Valenciennes, 1840. Eggs fertilised with *C. gariepinus* sperm. Asian Fish. Soc. 25, 270–277.
Olaniyi, W.A., Omitogun, O.G., 2013. Stages in the early and larval development of the African catfish *Clarias gariepinus* (Teleostei, Clariidae). Zygote 22, 314–330.
Olaniyi, W.A., Omitogun, O.G., 2014. Embryonic and larval developmental stages of African giant catfish *Heterobranchus bidorsalis* (Geoffroy Saint Hilaire, 1809) (Teleostei, Clariidae). SpringerPlus 3 (677), 1–15.
Puvaneswari, S., Kasi, M., Ramasamy, K., Mohammed, A.H., 2009. Early embryonic and larval development of Indian catfish, *Heteropneustes fossilis*. Eur. Asian J. Biol. Sci. 3, 84–96. https://doi.org/10.5053/ejobios.
Rahman, M.J., Wahab, Md.A., 2018. Catch trend and stock assessment of Hilsa *Tenualosa ilisha* using digital image measured length-frequency data. Mari. Coast. Fish. 10, 386–401. https://doi.org/10.1002/mcf2.10034.
Sahoo, A.K., Wahab, Md. Abdul, Phillips, M., Rahman, A., Padiyar, A., Puvanendran, V., Bangera, R., Belton, Ben, De, D.K., Meena, D.K., Behera, B.K., Sharma, A.K., Bhaumik, U., Mohanty, B.P., Choudhury, S.R., Mohan, C.V., 2016. Breeding and culture status of Hilsa (*Tenualosa ilisha*, Ham. 1822) in South Asia: a review. Rev. Aquac. 10 (1), 96–110. https://doi.org/10.1111/raq.12149.
Sen, P.R., De, D.K., Nath, D., 1990. Experiments on artificial propagation of Hilsa, *Tenualosa ilisha* (Hamilton). Ind. J. Fish. 37, 159–162.
Si, Luo., Benli, Wu, Xiaoqin, Xiong, Jianwei, Wang, 2016. Effects of total hardness and calcium: magnesium ratio of water during early stages of rare minnows (*Gobiocypris rarus*). Compar. Med. 66 (3), 181–187.
Silva, L.V.F., Golombieski, J.I., Baldisserotto, B., 2003. Incubation of silver catfish, *Rhamdia quelen* (Pimelodidae), eggs at different calcium and magnesium concentrations. Aquaculture 228, 279–287.
Silva, L.V.F., Golombieski, J.I., Baldisserotto, B., 2005. Growth and survival of silver catfish larvae, *Rhamdia quelen* (Heptapteridae), at different calcium and magnesium concentrations. Neotrop. Ichthyol. 3, 299–304.
Townsend, C.R., Silva, L.V.F., Baldisserotto, B., 2003. Growth and survival of *Rhamdia quelen* (Siluriformes, Pimelodidae) larvae exposed to different levels of water hardness. Aquaculture 215, 103–108.
Van der Velden, J.A., Spanings, F.A., Flik, G., Wendelaar Bonga, S.E., 1991. Early life stages of carp (*Cyprinus carpio* L.) depend on ambient magnesium for their development. J. Exp. Biol. 158, 431–438.
Wang, G.J., Xie, J., Pan, D.B., 2002. Effects of Ca2+, Mg2+ concentration and Ca2+/Mg2+ on hatching rates for the eggs of *Anguilla japonica* reared in artificial sea water. Mar. Sci. 26, 69–71.
Zhu, B.Q., Yu, J.J., Qin, X.G., Rioual, P., Xiong, H.G., 2012. Climatic and Geological Factors Contributing to the Natural Water Chemistry in an Arid Environment from Watersheds in Northern Xinxiang, China. https://doi.org/10.1016/j.geomorph.2012.02.014.